

1. A method of generating a proximal differentiated airway organoid (PD-organoid) comprising culturing an airway organoid (AO-organoid) in a proximal differentiation medium for a period of time sufficient to generate a PD-organoid comprising a cell population consisting of at least 25%, at least 30%, at least 35% or at least 40% ciliated cells, wherein the ciliated cells are characterised by FOXJ1 and SNTN expression.

2. The method of claim 1, wherein the proximal differentiation medium is supplemented with a notch inhibitor, optionally selected from the group consisting of a gamma-secretase inhibitor, such as DAPT or dibenzazepine (DBZ) or benzodiazepine (BZ) or LY-411575.

3. (canceled)

4. The method of claim 2, wherein the notch inhibitor is DAPT, preferably at a concentration of between 5 and 30 μ M, preferably between 10 and 20 μ M, or more preferably about 10 μ M.

5. The method of claim 1, wherein the proximal differentiation medium comprises one or more components as set out in Table 2, optionally at the concentrations shown in Table 2; and/or wherein the proximal differentiation medium is PneumaCult-ALI medium (StemCell Technologies) supplemented with notch inhibitor.

6. The method of claim 5, wherein the proximal differentiation medium comprises at least EGF, insulin, transferrin, hydrocortisone, triiodothyronine and epinephrine.

7. The method of claim 6, wherein the proximal differentiation medium further comprises bovine serum albumin and/or bovine pituitary extract.

8. (canceled)

9. The method of any claim 1, wherein the method further comprises one or more of the following steps prior to culturing the AO-organoid in a proximal differentiation medium:

- a. obtaining a lung tissue sample from a subject;
- b. obtaining dissociated cells from a lung tissue sample; and
- c. culturing lung cells in an AO-organoid formation phase for a period of time sufficient to generate an AO-organoid.

10. The method of claim 9, wherein the AO-organoid formation phase comprises culturing cells in an AO-organoid medium comprising one or more components as set out in Table 1, optionally at the concentrations shown in Table 1.

11. The method of claim 10, wherein the AO-organoid medium comprises at least R-spondin, a BMP inhibitor, a TGF-beta inhibitor, FGF and heregulin beta-1.

12. The method of claim 11, wherein the step of culturing the lung cells and/or AO-organoid comprises culturing the cells in contact with an exogenous extracellular matrix (such as a basement membrane extract or Matrigel™).

13. The method of claim 1, wherein: (a) the AO-organoid is a 3D organoid; (b) the PD-organoid is a 3D organoid; and/or (c) the PD-organoid is a 2D organoid.

14. (canceled)

15. (canceled)

16. The method of claim 13, wherein the step of culturing in a proximal differentiation medium comprises culturing in a transwell culture system comprising an apical and basal chamber.

17. A method of generating a 3D PD-organoid in accordance with claim 13 comprising the steps of:

a. culturing lung cells from a subject in an AO-organoid formation phase in an AO-organoid medium in contact with an extracellular matrix for a period of time sufficient to generate a 3D AO-organoid, for example for at least 2 days; and

b. changing the AO medium to a proximal differentiation medium supplemented with a notch inhibitor and culturing the 3D AO-organoid in the proximal differentiation medium supplemented with a notch inhibitor for a period of time sufficient to generate a PD-organoid, for example for at least 5 days, at least 10 days, at least 14 days or at least 16 days.

18. A method of generating a 2D PD-organoid in accordance with claim 13 comprising the steps of:

a. culturing lung cells from a subject in an AO-organoid formation phase in an AO-organoid medium in contact with an extracellular matrix for a period of time sufficient to generate a 3D AO-organoid, for example for at least 2 days;

b. dissociating the 3D AO-organoids into single cell suspension;

c. seeding the dissociated cells in the apical chamber of a transwell culture system;

d. optionally culturing the seeded cells in AO medium for at least 1 day, for example, until the cells reach at least 90% confluence; and

e. culturing the seeded cells in proximal differentiation medium supplemented with a notch inhibitor for a period of time sufficient to generate a 2D PD-organoid, for example for at least 5 days, at least 10 days, at least 14 days or at least 16 days.

19. The method of claim 16, wherein: (a) the culture medium is added to both the apical and basal chambers of the transwell culture system; (b) wherein the culture medium is refreshed every other day; and/or (c) the organoid or cells are human organoids or human cells.

20. (canceled)

21. (canceled)

22. A PD-organoid obtained by a method of claim 1, wherein the PD-organoid consists of a cell population comprising at least 25%, at least 30%, at least 35% or at least 40% ciliated cells, wherein the ciliated cells are characterised by FOXJ1 and SNTN expression.

23. The PD-organoid of claim 22, wherein: (a) the PD-organoid has at least 2-fold or at least 3-fold increase in the proportion of ciliated cells when compared to the AO-organoid from which it is derived; (b) the PD is further characterised by serine protease expression, for example, expression of one or more or all of TMPRSS2, TMPRSS4, TMPRSS11D (HAT) and Matriptase; (c) expression of HAT is at least 1 log₁₀ fold increased relative to its expression in AO-organoids; and/or (d) the ciliated cells make up at least 10-40% of the cells in the organoid by day 12, by day 14, or by day 16 after culturing in the proximal differentiation medium.

24. (canceled)

25. (canceled)

26. (canceled)